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The Atomic Composition of *Onoclea sensibilis* Spores

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It is generally accepted that fern spores contain all the elements that are needed for germination because spores germinate on distilled water in the absence of added nutrients (Raghavan, 1980). Here we present the first quantitative data on the atomic composition of *Onoclea* spores that permit one to know which elements are present in the spore. The presence of an element does not imply its essentiality as a nutrient; rather, it allows for the possibility that this element could be involved with development.

One element of particular interest to us is calcium, which is essential for spore germination (Wayne & Hepler, 1984a). Ninety percent of the calcium present in *Onoclea* spores resides in their walls, whereas 10% is intracellular (Wayne & Hepler, 1984b). By selectively removing calcium from the spores, we show that the wall-associated calcium is required for *Onoclea* germination in calcium-free media.

MATERIALS AND METHODS

Mature sporophylls of *Onoclea sensibilis* L. were collected in Amherst and Pelham, Massachusetts in January, 1981 and February, 1982 and stored in plastic bags in the freezer at −15°C. Prior to an experiment, sporangia were wetted with a 0.1% solution of Aerosol O. T. (Fisher Scientific Co., Pittsburgh, PA), sterilized for 1 min with 1 l of a 20% (v/v) solution of commercial bleach (5.25% NaOCl), and rinsed with 500 ml of sterile water in a manner modified from Stockwell and Miller (1974). For germination assays, approximately 2 mg of sterilized spores were sown on either 10 ml of the standard medium containing 1 mM $Ca(NO_3)_2$, 0.81 mM $MgSO_4$, and 3.45 mM KNO_3 (pH 5.2), or on 10 ml of 2.5 mM EGTA (ethylene glycol-bis(β-amino-ethyl ether)-N,N,N′,N′-tetraacetic acid, pH 6.8) for 24 h then transferred to 10 ml of the standard medium plus or minus $Ca(NO_3)_2$ one hour prior to irradiation. Spores were irradiated to induce germination for 5 minutes with broad band red light (energy fluence rate = 2.4 $J \cdot m^{-2} \cdot s^{-1}$). All manipulations were carried out in sterile plasticware in the dark or under a dim green safelight (Wayne & Hepler, 1984a).

Percent germination was determined 48 h after irradiation by the acetocarmine-chloral hydrate method of Edwards and Miller (1972). Data are expressed as the mean ± two standard errors of the mean.

In order to analyze the elements contained in the spores, sterilized spores were dried at 105°C for 2 h and weighed immediately. For the determination of calcium, magnesium, potassium, cobalt, nickel, iron, copper, manganese, sodium, zinc, mercury, cadmium, chromium, silver, molybdenum, vanadium, and lead,

spore samples were dissolved in hot concentrated sulfuric acid (1 ml) plus 10 drops of concentrated nitric acid, diluted with water to a 25–50 ml volume depending on weight (usually 10 mg), and then analyzed for metals using a Perkin-Elmer 403 Atomic Absorption Spectrophotometer following the Perkin-Elmer standard methods guide. Carbon, hydrogen, and nitrogen analyses were performed on a Perkin-Elmer 240 Elemental Analyser according to the modified Pregl-Dumas technique. Sulfur analysis was done by classical $BaSO_4$ titration using Thorin indicator after Schöniger oxygen flask combustion. Phosphorus was analyzed by forming phosphomolybdic acid from orthophosphate after conversion by hot H_2SO_4 and HNO_3 digestion. Phosphomolybdic acid was then measured spectrophotometrically at a wavelength of 882 nm. Oxygen analysis was done by a modified Unterzaucher procedure. Chlorine was analyzed as Cl^- after a Schöniger oxygen flask combustion followed by a coulometric titration with silver.

Culcita coniifolia, Cycas taiwaniana, Nothoscordum bivalve, Psilotum nudum, Selaginella kraussiana, and *Tradescantia virginiana* were grown in the greenhouse of the University of Massachusetts, Amherst. Voucher specimens are on deposit in the University of Massachusetts Herbarium, Amherst. *Funaria hygrometica* was grown in Laetsch's medium under continuous white light (Laetsch, 1967).

Results and Discussion

The atomic composition of spores of *Onoclea sensibilis* is shown in Table 1. The spores contain all the macro- and micronutrients needed for higher plant growth (Clarkson & Hanson, 1980). The relative atomic composition of *Onoclea* spores is similar to that reported for leaves of a variety of ferns (Höhne & Richter, 1981) and corn plants (Epstein, 1972). The high levels of minerals found in *Onoclea* spores contrast with the very low levels found in corn microspores (Pfahler & Linskins, 1973). The difference in the mineral content of corn microspores and *Onoclea* spores may reflect the functional differentiation of fern spores as propagules. *Onoclea* spores may carry sufficient nutrients to ensure the proper development of the gametophyte, whereas the style provides the nutrition for angiosperm microgametophytes. Consistent with their role as propagules, the spores can withstand considerable desiccation (cf. Barker & White, 1964) as evidenced by their low water content. In *Onoclea,* the dry weight accounts for 95.43 ± 1.02% of the fresh weight of the spore, whereas the dry weight of a plant cell may be only 7% of the fresh weight (Burling & Jackson, 1965; Ray, 1962).

Carbon, hydrogen, and oxygen are the most common elements in the spores. The carbon/hydrogen/oxygen molar ratio of 1/1.6/0.3 is a result of the abundance of storage lipids and proteins in the spores (DeMaggio & Stetler, 1980; Towill & Ikuma, 1975). This ratio remains nearly constant for 24 h following hydration. The carbon/hydrogen/oxygen ratio changes to 1/1.3/0.9 in week-old gametophytes indicating that in this later developmental stage carbon is mainly present as carbohydrate (Table 2).

Potassium, magnesium, sodium, and calcium are the major cations in the spores.

TABLE 1. The Atomic Composition of *Onoclea sensibilis* Spores.

Element	Percent dry weight	nmol/mg dry weight	Number of atoms relative to calcium
C	58.59 ± 2.00	48,784.35	977.64
O	21.25 ± 1.90	13,281.75	266.17
H	7.76 ± 0.55	76,942.16	1541.93
N	4.59 ± 0.28	3277.00	65.67
P	0.82 ± 0.18	254.74	5.31
K	0.70 ± 0.00	179.04	3.59
S	0.53 ± 0.07	163.76	3.28
Mg	0.34 ± 0.07	139.89	2.80
Na	0.23 ± 0.07	100.04	2.00
Ca	0.20 ± 0.07	49.90	1.00
Cl	0.110 ± 0.020	31.027	0.622
Co	0.040 ± 0.037	6.787	0.136
Fe	0.024 ± 0.006	4.297	0.086
Ni	0.011 ± 0.020	1.874	0.038
Pb	0.010 ± 0.018	0.483	0.010
Mn	0.008 ± 0.002	1.456	0.029
Zn	0.006 ± 0.003	0.918	0.018
Cu	0.005 ± 0.003	0.787	0.016
Cr	0.002 ± 0.000	0.385	0.008
Ag	0.002 ± 0.001	0.185	0.004
Cd	0.0004 ± 0.0006	0.035	0.0007
Hg	0.00001 ± 0.00001	0.0005	0.00001
V	<0.001	<0.196	<0.004
Mo	<0.0007	<0.073	<0.001
Unknown	4.8		
Total	100.0		

Calcium has been shown to be essential for the normal spore development. It is required for germination (Wayne & Hepler, 1984a), rhizoid growth (Miller et al., 1983), and normal protonema development (Cooke & Racusen, 1982). Calcium is also required for archegonium, antheridium, and sporophyte formation in *Woodsia obtusa* (Bryan & O'Kelley, 1967), and spermatogenesis in *Marsilea* (Wick, 1979). In *Onoclea*, the amount of calcium in the spores is similar to the levels of calcium found in the fiddlehead, expanded leaf, rachis, and empty sporangia; however, there is considerably more calcium found in gametophytes growing in a calcium-sufficient medium (Table 3). The average calcium content in the various tissues of *Onoclea* is 0.24% which is similar to that found in a variety of greenhouse-grown plants (Table 3).

Calcium, primarily localized in spore walls, is necessary for germination (Wayne & Hepler, 1984b). *Onoclea* spores, for example, germinate readily when placed in deionized water plus $MgSO_4$ and KNO_3, but not if they are prewashed with EGTA, a chelating agent that removes the wall bound calcium. The ability to germinate can be regained by adding exogenous calcium (Table 4), indicating the importance of external calcium. The potential of unwashed spores to germinate in calcium-free media depends on the spore density. We have found that

TABLE 2. The Carbon, Hydrogen, and Oxygen Ratios of Several Developmental Stages of *Onoclea*.

Developmental stage	Element	Atomic content (μmol/mg dry wt)	Ratio with respect to carbon
Dry spores	C	48.78 ± 1.66	1
	H	76.94 ± 5.46	1.6
	O	13.28 ± 1.19	0.3
Hydrated spores (dark)	C	48.40 ± 1.77	1
	H	81.56 ± 3.37	1.7
	O	15.00 ± 0.13	0.3
Hydrated spores (5 min red)	C	49.07 ± 1.03	1
	H	81.61 ± 1.49	1.7
	O	13.91 ± 1.44	0.3
Gametophytes (1 week old)	C	34.03 ± 3.36	1
	H	44.55 ± 2.56	1.3
	O	29.75 ± 9.75	0.9

germination is promoted by high spore density, probably as a result of pooling sufficient calcium from many cell walls (Wayne & Hepler, 1984a). Other studies also show that plants are capable of adding nutrients to "nutrient free," media thereby masking the requirement for a given nutrient (Miller et al., 1983; Pfahler & Linskins, 1973; Saunders & Hepler, 1983; Wayne & Hepler, 1984a).

The concentrations of the transition metals cobalt, nickel, lead, chromium, silver, and copper are relatively high (cf. Gauch, 1972). These trace elements may accumulate in the spores in order to provide a reservoir for the growing

TABLE 3. The Calcium Content of Plant Tissues.

Species	Tissue or organ	Calcium content (% dry weight)
Onoclea sensibilis	gametophyte	0.46
	empty sporangia	0.26
	rachis	0.22
	spore	0.20[a]
	expanded leaf	0.17
	fiddlehead	0.11
Onoclea sensibilis	average of all parts	0.24
Psilotum nudum	frond	0.06
Culcita coniifolia	frond	0.12
Selaginella kraussiana	shoot	0.42
Cycas taiwaniana	leaf	0.47
Nothoscordum bivalve	leaf	0.52
Funaria hygrometica	protonema	0.64
Tradescantia virginiana	leaf	1.10[b]

[a] The range is 0.1–0.4% dry weight.

[b] The high calcium content of *T. virginiana* is a consequence of the presence of calcium oxalate crystals.

TABLE 4. The Effect of Washing with EGTA on Germination of *Onoclea*.

Treatment	Percent germination
Control	89.00 ± 1.00
EGTA-washed	0.75 ± 1.50
EGTA-washed + 1 mM $Ca(NO_3)_2$	87.25 ± 4.50

gametophyte and young sporophyte before a sufficiently large root system can be produced. Alternatively, the accumulation of these heavy metals may be fortuitous but not essential (especially in the case of cobalt), indicating that *Onoclea* may be a bio-accumulator of heavy metals from the environment and thus may serve as a bio-indicator. Other ferns have been shown to accumulate heavy metals from the environment (see Puckett & Burton, 1981, for a review; Hunter, 1953). This would provide yet a new way in which a fern can be utilized as an environmental monitor (Klekowski & Poppel, 1976; Petersen et al., 1980; Petersen & Francis, 1980). Interestingly, many of these bivalent metals can act as calcium antagonists (Hagiwara, 1973) and thus as inhibitors of germination (Petersen et al., 1980; Petersen & Francis, 1980; Wayne & Hepler, 1984a).

The concentration of chloride is too low for it to serve as the only counter ion for potassium. The positive charges are probably neutralized by organic acids such as malic acid, which would account for the low intracellular pH we have observed in *Onoclea*.

The high level of phosphorus is probably due to the presence of phytic acid (inositol hexaphosphate) in the spores (DeMaggio et al., 1983). Inositol polyphosphate is usually considered to be a storage form of phosphate, although recently it has been shown to interact with calcium ions in triggering cell responses (Rasmussen, 1981).

Silicon is present in high concentrations in the spores of *Selaginella* (Tryon & Lugardon, 1978) and the leaves of many true ferns (Höhne & Richter, 1981). It may also be part of the spores of *Onoclea*. We, however, were unable to assay for it.

Here we have presented the natural abundance of elements in the spores of *Onoclea*. We have shown how the C/H/O ratio in particular reveals the developmental state of the cells and that there is sufficient calcium associated with the cells to facilitate germination. By removing the wall-bound calcium, we have also shown that the germination response requires external calcium. A bio-inorganic chemical analysis of spores complements other methods of analysis such as light (Vogelmann & Miller, 1980) and electron (Bassel et al., 1981) microscopy, biochemical studies (Huckaby & Miller, 1984; Towill & Ikuma, 1975; DeMaggio & Stetler, 1980) as well as physiological investigations (Vogelmann et al., 1981; Wayne & Hepler, 1984a), in understanding *Onoclea* spores and their development.

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REVIEW

"*Gametophytes of Ophioglossaceae*," by D. D. Pant, D. D. Nautiyal, and D. R. Misra. Phyta Monograph 1:1–111, 1984.

This is a report on the gametophytes and young embryos of the Ophioglossaceae in India. Six species of *Ophioglossum*, 4 species of *Botrychium*, and *Helminthostachys* were studied from extensive collections of gametophytes. A helpful introduction to the literature initiates this conventional morphological study. Observations on the structure and development of these gametophytes and embryos expand our knowledge about the gametophytes from India. New and interesting information presented includes: meristems of the gametophyte lobes of *Helminthostachys* that suggest that the lobes are short lateral branches; slight lignification of the central strand of elongated parenchyma cells in the cylindrical gametophytes of *Ophioglossum* and *Helminthostachys*; absence of a ventral canal cell in *Ophioglossum* archegonia; and endoscopic embryo development in *O. nudicaule*. The concluding section of the monograph summarizes the new observations and incorporates them into a useful review of the literature. Pertinent information from the literature is also tabulated in a 12 page table at the end of the text. There is an abundance of illustrations (over half of the monograph). The line drawings are excellent but the halftones are in need of improvement. The quality of the halftones is not a serious detraction from the paper because much of the illustrated material is presented as both line drawings and halftones. "*Gametophytes of Ophioglossaceae*" is an informative paper that will be of interest to pteridologists, especially those interested in the morphology and anatomy of gametophytes. This monograph may be purchased from the Society of Indian Plant Taxonomists, Department of Botany, University of Allahabad, Allahabad 211002, India.—DEAN P. WHITTIER, Department of Biology, Vanderbilt University, Nashville, TN 37235.